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(54) **Human 7-transmembrane receptor HLWAR77**

(57) HLWAR77 polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing HLWAR77 polypeptides and polynucleotides in the design of protocols for the treatment of infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; diabetes, obesity; anorexia; bulimia; asthma; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hypertrophy; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette's syndrome, among others and diagnostic assays for such conditions.

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Description

This application claims the benefit of U.S. Provisional Application Nos. 60/049,332, filed June 11, 1997, and 60/067,253, filed December 2, 1997, which are herein incorporated by reference in their entirety.

FIELD OF INVENTION

This invention relates to newly identified polynucleotides, polypeptides encoded by them and to the use of such polynucleotides and polypeptides, and to their production. More particularly, the polynucleotides and polypeptides of the present invention relate to G-protein coupled receptor family, hereinafter referred to as HLWAR77. The invention also relates to inhibiting or activating the action of such polynucleotides and polypeptides.

BACKGROUND OF THE INVENTION

It is well established that many medically significant biological processes are mediated by proteins participating in signal transduction pathways that involve G-proteins and/or second messengers, e.g., cAMP (Lefkowitz, *Nature*, 1991, 351:353-354). Herein these proteins are referred to as proteins participating in pathways with G-proteins or PPG proteins. Some examples of these proteins include the GPC receptors, such as those for adrenergic agents and dopamine (Kobilka, B.K., et al., *Proc. Natl Acad. Sci., USA*, 1987, 84:46-50; Kobilka, B.K., et al., *Science*, 1987, 238:650-656; Bunzow, J.R., et al., *Nature*, 1988, 336:783-787), G-proteins themselves, effector proteins, e.g., phospholipase C, adenylyl cyclase, and phosphodiesterase, and actuator proteins, e.g., protein kinase A and protein kinase C (Simon, M.I., et al., *Science*, 1991, 252:802-8).

For example, in one form of signal transduction, the effect of hormone binding is activation of the enzyme, adenylyl cyclase, inside the cell. Enzyme activation by hormones is dependent on the presence of the nucleotide, GTP. GTP also influences hormone binding. A G-protein connects the hormone receptor to adenylyl cyclase. G-protein was shown to exchange GTP for bound GDP when activated by a hormone receptor. The GTP-carrying form then binds to activated adenylyl cyclase. Hydrolysis of GTP to GDP, catalyzed by the G-protein itself, returns the G-protein to its basal, inactive form. Thus, the G-protein serves a dual role, as an intermediate that relays the signal from receptor to effector, and as a clock that controls the duration of the signal.

The membrane protein gene superfamily of G-protein coupled receptors has been characterized as having seven putative transmembrane domains. The domains are believed to represent transmembrane α -helices connected by extracellular or cytoplasmic loops. G-protein coupled receptors include a wide range of biologically active receptors, such as hormone, viral, growth factor and neuroreceptors.

G-protein coupled receptors (otherwise known as 7TM receptors) have been characterized as including these seven conserved hydrophobic stretches of about 20 to 30 amino acids, connecting at least eight divergent hydrophilic loops. The G-protein family of coupled receptors includes dopamine receptors which bind to neuroleptic drugs used for treating psychotic and neurological disorders. Other examples of members of this family include, but are not limited to, calcitonin, adrenergic, endothelin, cAMP, adenosine, muscarinic, acetylcholine, serotonin, histamine, thrombin, kinin, follicle stimulating hormone, opsins, endothelial differentiation gene-1, rhodopsins, odorant, and cytomegalovirus receptors.

Most G-protein coupled receptors have single conserved cysteine residues in each of the first two extracellular loops which form disulfide bonds that are believed to stabilize functional protein structure. The 7 transmembrane regions are designated as TM1, TM2, TM3, TM4, TM5, TM6, and TM7. TM3 has been implicated in signal transduction.

Phosphorylation and lipidation (palmitoylation or farnesylation) of cysteine residues can influence signal transduction of some G-protein coupled receptors. Most G-protein coupled receptors contain potential phosphorylation sites within the third cytoplasmic loop and/or the carboxy terminus. For several G-protein coupled receptors, such as the β -adrenoreceptor, phosphorylation by protein kinase A and/or specific receptor kinases mediates receptor desensitization.

For some receptors, the ligand binding sites of G-protein coupled receptors are believed to comprise hydrophilic sockets formed by several G-protein coupled receptor transmembrane domains, said sockets being surrounded by hydrophobic residues of the G-protein coupled receptors. The hydrophilic side of each G-protein coupled receptor transmembrane helix is postulated to face inward and form a polar ligand binding site. TM3 has been implicated in several G-protein coupled receptors as having a ligand binding site, such as the TM3 aspartate residue. TM5 serines, a TM6 asparagine and TM6 or TM7 phenylalanines or tyrosines are also implicated in ligand binding.

G-protein coupled receptors can be intracellularly coupled by heterotrimeric G-proteins to various intracellular enzymes, ion channels and transporters (see, Johnson et al., *Endoc. Rev.*, 1989, 10:317-331). Different G-protein α -subunits preferentially stimulate particular effectors to modulate various biological functions in a cell. Phosphorylation of cytoplasmic residues of G-protein coupled receptors has been identified as an important mechanism for the regula-

tion of G-protein coupling of some G-protein coupled receptors. G-protein coupled receptors are found in numerous sites within a mammalian host.

Over the past 15 years, nearly 350 therapeutic agents targeting 7 transmembrane (7 TM) receptors have been successfully introduced onto the market.

This indicates that these receptors have an established, proven history as therapeutic targets. Clearly there is a need for identification and characterization of further receptors which can play a role in preventing, ameliorating or correcting dysfunctions or diseases, including, but not limited to, infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; diabetes, obesity; anorexia; bulimia; asthma; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hypertrophy; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette's syndrome.

SUMMARY OF THE INVENTION

In one aspect, the invention relates to HLWAR77 polypeptides and recombinant materials and methods for their production. Another aspect of the invention relates to methods for using such HLWAR77 polypeptides and polynucleotides. Such uses include the treatment of infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; diabetes, obesity; anorexia; bulimia; asthma; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hypertrophy; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette's syndrome, among others. In still another aspect, the invention relates to methods to identify agonists and antagonists using the materials provided by the invention, and treating conditions associated with HLWAR77 imbalance with the identified compounds. Yet another aspect of the invention relates to diagnostic assays for detecting diseases associated with inappropriate HLWAR77 activity or levels.

DESCRIPTION OF THE INVENTION

Definitions

The following definitions are provided to facilitate understanding of certain terms used frequently herein.

"HLWAR77" refers, among others, to a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2, or an allelic variant thereof.

"Receptor Activity" or "Biological Activity of the Receptor" refers to the metabolic or physiologic function of said HLWAR77 including similar activities or improved activities or these activities with decreased undesirable side-effects. Also included are antigenic and immunogenic activities of said HLWAR77.

"HLWAR77 gene" refers to a polynucleotide comprising the nucleotide sequence set forth in SEQ ID NO:1 or allelic variants thereof and/or their complements.

"Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library.

"Isolated" means altered "by the hand of man" from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

"Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications has been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide

bonds or modified peptide bonds, i.e., peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993 and Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter *et al.*, "Analysis for protein modifications and nonprotein cofactors", *Meth Enzymol* (1990) 182:626-646 and Rattan *et al.*, "Protein Synthesis: Posttranslational Modifications and Aging", *Ann NY Acad Sci* (1992) 663:48-62.

"Variant" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Identity" is a measure of the identity of nucleotide sequences or amino acid sequences. In general, the sequences are aligned so that the highest order match is obtained. "Identity" *per se* has an art-recognized meaning and can be calculated using published techniques. See, e.g.: (COMPUTATIONAL MOLECULAR BIOLOGY, Lesk, A.M., ed., Oxford University Press, New York, 1988; BIOCOMPUTING: INFORMATICS AND GENOME PROJECTS, Smith, D.W., ed., Academic Press, New York, 1993; COMPUTER ANALYSIS OF SEQUENCE DATA, PART I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; SEQUENCE ANALYSIS IN MOLECULAR BIOLOGY, von Heinje, G., Academic Press, 1987; and SEQUENCE ANALYSIS PRIMER, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073. Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCS program package (Devereux, J., *et al.*, *Nucleic Acids Research* (1984) 12(1):387), BLASTP, BLASTN, FASTA (Atschul, S.F. *et al.*, *J Molec Biol* (1990) 215:403).

As an illustration, by a polynucleotide having a nucleotide sequence having at least, for example, 95% "identity" to a reference nucleotide sequence of SEQ ID NO: 1 is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence of SEQ ID NO: 1. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the

reference sequence may occur at the 5 or 3 terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

Similarly, by a polypeptide having an amino acid sequence having at least, for example, 95% "identity" to a reference amino acid sequence of SEQ ID NO:2 is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of SEQ ID NO: 2. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

Polypeptides of the Invention

In one aspect, the present invention relates to HLWAR77 polypeptides (or HLWAR77 proteins). The HLWAR77 polypeptides include the polypeptides of SEQ ID NOS:2 and 4; as well as polypeptides comprising the amino acid sequence of SEQ ID NO:2; and polypeptides comprising the amino acid sequence which have at least 80% identity to that of SEQ ID NO:2 over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO: 2. Furthermore, those with at least 97-99% are highly preferred. Also included within HLWAR77 polypeptides are polypeptides having the amino acid sequence which have at least 80% identity to the polypeptide having the amino acid sequence of SEQ ID NO: 2 over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO: 2. Furthermore, those with at least 97-99% are highly preferred. Preferably HLWAR77 polypeptides exhibit at least one biological activity of the receptor.

The HLWAR77 polypeptides may be in the form of the "mature" protein or may be a part of a larger protein such as a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production.

Fragments of the HLWAR77 polypeptides are also included in the invention. A fragment is a polypeptide having an amino acid sequence that entirely is the same as part, but not all, of the amino acid sequence of the aforementioned HLWAR77 polypeptides. As with HLWAR77 polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, and 101 to the end of HLWAR77 polypeptide. In this context "about" includes the particularly recited ranges larger or smaller by several, 5, 4, 3, 2 or 1 amino acid at either extreme or at both extremes.

Preferred fragments include, for example, truncation polypeptides having the amino acid sequence of HLWAR77 polypeptides, except for deletion of a continuous series of residues that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus or deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Also preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Other preferred fragments are biologically active fragments. Biologically active fragments are those that mediate receptor activity, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those that are antigenic or immunogenic in an animal, especially in a human.

Preferably, all of these polypeptide fragments retain the biological activity of the receptor, including antigenic activity. Among the most preferred fragment is that having the amino acid sequence of SEQ ID NO: 4. Variants of the defined sequence and fragments also form part of the present invention. Preferred variants are those that vary from the references by conservative amino acid substitutions -- i.e., those that substitute a residue with another of like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination.

The HLWAR77 polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

Polynucleotides of the Invention

Another aspect of the invention relates to HLWAR77 polynucleotides. HLWAR77 polynucleotides include isolated polynucleotides which encode the HLWAR77 polypeptides and fragments, and polynucleotides closely related thereto. More specifically, HLWAR77 polynucleotide of the invention include a polynucleotide comprising the nucleotide sequence contained in SEQ ID NO:1 encoding a HLWAR77 polypeptide of SEQ ID NO: 2, and polynucleotides having the particular sequences of SEQ ID NOS:1 and 3. HLWAR77 polynucleotides further include a polynucleotide comprising a nucleotide sequence that has at least 80% identity over its entire length to a nucleotide sequence encoding the HLWAR77 polypeptide of SEQ ID NO:2, and a polynucleotide comprising a nucleotide sequence that is at least 80% identical to that of SEQ ID NO:1 over its entire length. In this regard, polynucleotides at least 90% identical are particularly preferred, and those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred and those with at least 98-99% are most highly preferred, with at least 99% being the most preferred. Also included under HLWAR77 polynucleotides are a nucleotide sequence which has sufficient identity to a nucleotide sequence contained in SEQ ID NO:1 to hybridize under conditions useable for amplification or for use as a probe or marker. The invention also provides polynucleotides which are complementary to such HLWAR77 polynucleotides.

HLWAR77 of the invention is structurally related to other proteins of the G-protein coupled receptor family, as shown by the results of sequencing the cDNA encoding human HLWAR77. The cDNA sequence of SEQ ID NO:1 contains an open reading frame (nucleotide number 348 to 1610) encoding a polypeptide of 421 amino acids of SEQ ID NO:2. The amino acid sequence of Table 1 (SEQ ID NO:2) has about 33% identity (using FASTA) in 300 amino acid residues with Neuropeptide Y receptor. Li X., Wu Y, North R., Forte M. 1992. J. Biol. Chem., 267, 9-12. The nucleotide sequence of Table 1 (SEQ ID NO:1) has about 50% identity (using FASTA) in 730 nucleotide residues with Neuropeptide Y receptor. Li X, Wu Y, North R., Forthe M. 1992. J. Biol. Chem. 267, 9-122. Thus, HLWAR77 polypeptides and polynucleotides of the present invention are expected to have, inter alia, similar biological functions/properties to their homologous polypeptides and polynucleotides, and their utility is obvious to anyone skilled in the art.

Table 1^a

1	CCATCCTAAT ACGACTCACT ATAGGGCTCG AGCGGCCGCC CGGGCAGGTT
51	CTCGGCTCAC TGCAAGCTCC ACCTCCTGGG TTCACGCTAT TCTCCTGCCT
101	CAGCCTCCTG AGTAGCTGGG ACTACAGGCG CCCGCCACCA CGCCTGGCTA
151	ATTTTTTTtGT ATTTTtAGTa GGGACGGCGT TTCACTGTGT TAGCCCAGAT
201	GGTcTCCGTC TCCcGACCTc GtGATCCACC CACcTCGGCT TCCCAAAGTG
251	CTGGGATTAC AGGCGTGAGC CACCGCGCCC GGCCAATTTC CTTTCTTAAT

5 301 TGCCTctGCC CACCTCTTCT cTTCTGCTTC CATATTACAG GTTCATCATG

351 AATGAGAAAT GGGACACAAA CTCTTCAGAA AACTGGCATC CCATCTGGAA

10 401 TGTCAATGAC ACAAAAGCATC ATCTGTACTC AGATATTAAT ATTACCTATG

451 TGAACTACTA TcTTCACCAG CCTCAAGTGG CAGCAATCTT CATTATTTCC

15 501 TACTTTCTGA TCTTCTTTTT GTGCATGATG GGAATACTG TGGTTTGCTT

551 TATTGTAATG AGGAACAAAC ATATGCACAC AGTCACTAAT CTCTTCATCT

20 601 TAAACCTGGCCATAAGTGAT TTACTAGTTG GCATATTCTG CATGCCTATA

651 ACACTGCTGG ACAATATTAT AGCAGGATGG CCATTGGAA ACACGATGTG

25 701 CAAGATCAGT GGATTGGTCC AGGGAATATC TGTGCGAGCT TCAGTCTTTA

751 CGTTAGTTGC AATTGCTGTA GATAGGTTCC AGTGTGTGGT CTACCCTTTT

30 801 AAACCAAAGC TCACTATCAA GACAGCGTTT GTCATTATTA TGATCATCTG

851 GGTCCTAGCC ATCACCATTA TGTCTCCATC TGCAGTAATG TTACATGTGC

35 901 AAGAAGAAAA ATATTACCGA GTGAGACTCA ACTCCCAGAA TAAAACCACT

951 CCAGTCTACT GGTGCCGGGA AGACTGGCCA AATCAGGAAA TGAGGAAGAT

40 1001 CTACACCACT GTGCTGTTTG CCAACATCTA CCTGGCTCCC CTCTCCCTCA

1051 TTGTCATCAT GTATGGAAGG ATTGGAATTT CACTCTTCAG GGCTGCAGTT

45 1101 CCTCACACAG GCAGGAAGAA CCAGGAGCAG TGGCACGTGG TGTCCAGGAA

1151 GAAGCAGAAG ATCATTAAAG TGCTCCTGAT TGTGGCCCTG CTTTTTATTC

50 1201 TCTCATGGCT GCCCCTGTGG ACTCTAATGA TGCTCTCAGA CTACGCTGAC

1251 CTTTCTCCAA ATGAACTGCA GATCATCAAC ATCTACATCT ACCCTTTTGC

251 VPHTGRKNQE QWHVVSRRKQ KIIKMLLIVA LLFILSWLPL WTLMLSDYA
 301 DLSPNELQII NIYYPPFAHW LAFGNSSVNP IIYGFFNENF RRGFQEAFL
 351 QLCQKRAKPM EAYALKAKSH VLINTSNQLV QESTFQNPFG ETLLYRKS
 401 KPQQELVMEE LKETTNSSEI *

^b An amino acid sequence of a human HLWAR77 (SEQ ID NO: 2).

One polynucleotide of the present invention encoding HLWAR77 may be obtained using standard cloning and screening, from a cDNA library derived from mRNA in cells of Human placenta using the expressed sequence tag (EST) analysis (Adams, M.D., *et al. Science* (1991) 252:1651-1656; Adams, M.D. *et al., Nature*, (1992) 355:632-634; Adams, M.D., *et al., Nature* (1995) 377 Supp:3-174). Polynucleotides of the invention can also be obtained from natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

The nucleotide sequence encoding HLWAR77 polypeptide of SEQ ID NO:2 may be identical to the polypeptide encoding sequence contained in Table 1 (nucleotide number 348 to 1610 of SEQ ID NO:1), or it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2.

When the polynucleotides of the invention are used for the recombinant production of HLWAR77 polypeptide, the polynucleotide may include the coding sequence for the mature polypeptide or a fragment thereof, by itself; the coding sequence for the mature polypeptide or fragment in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be encoded. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Geritz *et al., Proc Natl Acad Sci USA* (1989) 86:821-824, or is an HA tag. The polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

Further preferred embodiments are polynucleotides encoding HLWAR77 variants comprising the amino acid sequence of HLWAR77 polypeptide of Table 2 (SEQ ID NO:2) in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acid residues are substituted, deleted or added, in any combination. Among the preferred polynucleotides of the present invention is contained in Table 3 (SEQ ID NO: 3) encoding the amino acid sequence of Table 4 (SEQ ID NO: 4).

Table 3²

1 AGTGAACCGT CAGATCGCCT GGAGACGCCA TCCACGCTGT TTTGACCTCC
 51 ATAGAAGACA CCGGGACCGA TCCAGCCTCC GGACTCTAGC CTAGGCCGCG
 101 GGACGGATAA CAATTTTACA CAGGAAACAG CTATGACCAC TAGGCTTTTG
 151 CAAAAAGCTA TTTAGGTGAC ACTATAGAAG GTACGCCTGC AGGTACCGGT
 201 CCGGAATTCC CGGGTCGACC CACGCGTCCG CATAAGTGAT TTACTAGTTG
 251 GCATATTCTG CATGCCTATA ACACTGCTGG ACAATATTAT AGCAGGATGG
 301 CCATTTGGAA ACACGATGTG CAAGATCAGT GGATTGGTCC AGGGAATATC
 351 TGTGCGAGCT TCAGTCTTTA CGTTAGTTGC AATTGCTGTA GATAGGTTCC
 401 AGTGTGTGGT CTACCCTTTT AAACCAAAGC TCACTATCAA GACAGCGTTT
 451 GTCATTATTA TGATCATCTG GGTCCTAGCC ATCACCATTA TGTCTCCATC
 501 TGCAGTAATG TTACATGTGC AAGAAGAAAA ATATTACCGA GTGAGACTCA
 551 ACTCCCAGAA TAAAACCACT CCACTCTACT GGTGCCGGGA AGACTGGCCA
 601 AATCAGGAAA TGAGGAAGAT CTACACCACT GTGCTGTTTG CCAACATCTA
 651 CCTGGCTCCC CTCTCCCTCA TTGTCATCAT GTATGGAAGG ATTGGAATTT
 701 CACTCTTCAG GGCTGCAGTT CCTCACACAG GCAGGAAGAA CCAGGAGCAG
 751 TGGCACGTGG TGTCCAGGAA GAAGCAGAAG ATCATTAGA TGCTCCTGAT
 801 TGTGGCCCTG CTTTTTATTC TCTCATGGCT GCCCCTGTGG ACTCTAATGA
 851 TGCTCTCAGA CTACGCTGAC CTTTCTCCAA ATGAACTGCA GATCATCAAC
 901 ATCTACATCT ACCCTTTTGC AACTGGCTG GCATTCGGCA ACAGCAGTGT
 951 CAATCCCATC ATTTATGGTT TCTTCAACGA GAATTTCCGC CGTGGTTTCC

1001 AAGAAGCTTT CCAGCTCCAG CTCTGCCAAA AAAGAGCAAA GCCTATGGAA
 1051 GCTTATGCCC TAAAAGCTAA AAGCCATGTG CTCATAAACA CATCTAATCA
 1101 GCTTGTCCAG GAATCTACAT TTCAAAACCC TCATGGGGAA ACCTTGCTTT
 1151 ATAGGAAAAG TGCTGAAAAA CCCCAACAGG AATTAGTGAT GGAAGAATTA
 1201 AAAGAAACTA CTAACAGCAG TGAGATTTAA AAAGAGCTAG TGTGATAATC
 1251 CTAACCTCTAC TACGCATTAT ATATTTAAAT CCATTGCTTT TTGTGGCTTT
 1301 GCACTTCAAA TTTTTCAAAG AATGTTCTAA ATAAAACATT TACTGAAAGC
 1351 CCTCTCTGGC AAAAAAATTA AAAATAAACA AAAATGGTCA TAAGATCATA
 1401 AACAACTCTTA TGTTGTATAA AAATACGTAG AGTGACTTAG ACATGTTTGC
 1451 ATGAATAAAT ATATTTCTAG AGAACAGTTA AAAAAAAAAA AAAAAAAAAA
 1501 AAAAAAAAA

* A partial nucleotide sequence of a human HLWAR77 (SEQ ID NO: 3).

Table 4¹

1 PRVRISDLLV GIFCMPITLL DNIIAGWPFG NTMCKISGLV QGISVAASVF
 51 TLVAIAVDRF QCVVYPFKPK LTIKTAHVII MIIWVLAITI MSPSAVMLHV
 101 QBEKYRVRL NSQNKTSFVY WCREDWPNQE MRKIYTTVLF ANIYLAPLSL
 151 IVIMYGRIGI SLFRAAVPHT GRKNQEQWHV VSRKKQKIIK MLLIVALLFI
 201 LSWLPLWTLM MLSDYADLSP NELQIINIYI YPFAHWLAFG NSSVNPIIYG
 251 PFNENFRRGF QEAFQLQLCQ KRAKPMAYYA LKAKSHVLIN TSNQLVQEST
 301 FQNPHEGTELL YRKSAEKPQQ ELVMEELKET TNSSEI*

^d A partial amino acid sequence of a human HLWAR77 (SEQ ID NO: 4).

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The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 80%, and preferably at least 90%, and more preferably at least 95%, yet even more preferably 97-99% identity between the sequences.

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Polynucleotides of the invention, which are identical or sufficiently identical to a nucleotide sequence contained in SEQ ID NO:1 or a fragment thereof may be used as hybridization probes for cDNA and genomic DNA, to isolate full-length cDNAs and genomic clones encoding HLWAR77 and to isolate cDNA and genomic clones of other genes (including genes encoding homologs and orthologs from species other than human) that have a high sequence similarity to the HLWAR77 gene. Such hybridization techniques are known to those of skill in the art. Typically these nucleotide sequences are 80% identical, preferably 90% identical, more preferably 95% identical to that of the referent. The probes generally will comprise at least 15 nucleotides. Preferably, such probes will have at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will range between 30 and 50 nucleotides.

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In one embodiment, to obtain a polynucleotide encoding HLWAR77 polypeptide, including homologs and orthologs from species other than human comprises the steps of screening an appropriate library under stringent hybridization conditions with a labeled probe having the SEQ ID NO: 1 or a fragment thereof (including that of SEQ ID NO: 3), and isolating full-length cDNA and genomic clones containing said polynucleotide sequence. Such hybridization techniques are well known to those of skill in the art. Stringent hybridization conditions are as defined above or alternatively conditions under overnight incubation at 42°C in a solution comprising: 50% formamide, 5xSSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10 % dextran sulfate, and 20 microgram/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

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The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments and diagnostics to animal and human disease.

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Vectors, Host Cells, Expression

The present invention also relates to vectors which comprise a polynucleotide or polynucleotides of the present invention, and host cells which are genetically engineered with vectors of the invention and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

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For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention. Introduction of polynucleotides into host cells can be effected by methods described in many standard laboratory manuals, such as Davis et al., *BASIC METHODS IN MOLECULAR BIOLOGY* (1986) and Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) such as calcium phosphate transfection, DEAE-dextran mediated transfection, transfection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection.

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Representative examples of appropriate hosts include bacterial cells, such as streptococci, staphylococci, *E. coli*, *Streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, HEK 293 and Bowes melanoma cells; and plant cells.

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A great variety of expression systems can be used. Such systems include, among others, chromosomal, episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL* (*supra*).

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For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or

into the extracellular environment, appropriate secretion signals may be incorporated into the desired polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

If the HLWAR77 polypeptide is to be expressed for use in screening assays, generally, it is preferred that the polypeptide be produced at the surface of the cell. In this event, the cells may be harvested prior to use in the screening assay. If HLWAR77 polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the polypeptide; if produced intracellularly, the cells must first be lysed before the polypeptide is recovered.

HLWAR77 polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

Diagnostic Assays

This invention also relates to the use of HLWAR77 polynucleotides for use as diagnostic reagents. Detection of a mutated form of HLWAR77 gene associated with a dysfunction will provide a diagnostic tool that can add to or define a diagnosis of a disease or susceptibility to a disease which results from under-expression, over-expression or altered expression of HLWAR77. Individuals carrying mutations in the HLWAR77 gene may be detected at the DNA level by a variety of techniques.

Nucleic acids for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar fashion. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled HLWAR77 nucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. See, e.g., Myers *et al.*, *Science* (1985) 230:1242. Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method. See Cotton *et al.*, *Proc Natl Acad Sci USA* (1985) 85: 4397-4401. In another embodiment, an array of oligonucleotides probes comprising HLWAR77 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability. (See for example: M.Chee *et al.*, *Science*, Vol 274, pp 610-613 (1996)).

The diagnostic assays offer a process for diagnosing or determining a susceptibility to infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; diabetes, obesity; anorexia; bulimia; asthma; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hypertrophy; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette's syndrome through detection of mutation in the HLWAR77 gene by the methods described.

In addition, infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; diabetes, obesity; anorexia; bulimia; asthma; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hypertrophy; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette's syndrome, can be diagnosed by methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of HLWAR77 polypeptide or HLWAR77 mRNA. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as an HLWAR77, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

Thus in another aspect, the present invention relates to a diagnostic kit for a disease or susceptibility to a disease, particularly infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; diabetes, obesity; anorexia; bulimia; asthma; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; aller-

gies; benign prostatic hypertrophy; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette's syndrome, which comprises:

- (a) a HLWAR77 polynucleotide, preferably the nucleotide sequence of SEQ ID NO: 1, or a fragment thereof;
- (b) a nucleotide sequence complementary to that of (a);
- (c) a HLWAR77 polypeptide, preferably the polypeptide of SEQ ID NO: 2, or a fragment thereof; or
- (d) an antibody to a HLWAR77 polypeptide, preferably to the polypeptide of SEQ ID NO: 2. It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

Chromosome Assays

The nucleotide sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important first step in correlating those sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

The differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

Antibodies

The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them can also be used as immunogens to produce antibodies immunospecific for the HLWAR77 polypeptides. The term "immunospecific" means that the antibodies have substantial greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

Antibodies generated against the HLWAR77 polypeptides can be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., *Nature* (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, *Immunology Today* (1983) 4:72) and the EBV-hybridoma technique (Cole *et al.*, MONOCLONAL ANTIBODIES AND CANCER THERAPY, pp. 77-96, Alan R. Liss, Inc., 1985).

Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms including other mammals, may be used to express humanized antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.

Antibodies against HLWAR77 polypeptides may also be employed to treat infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; diabetes; obesity; anorexia; bulimia; asthma; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hypertrophy; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette's syndrome, among others.

Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in a mammal which comprises inoculating the mammal with HLWAR77 polypeptide, or a fragment thereof, adequate to produce antibody and/or T cell immune response to protect said animal from infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; diabetes; obesity; anorexia; bulimia; asthma; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hypertrophy; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias,

such as Huntington's disease or Gilles de la Tourette's syndrome, among others. Yet another aspect of the invention relates to a method of inducing immunological response in a mammal which comprises, delivering HLWAR77 polypeptide via a vector directing expression of HLWAR77 polynucleotide *in vivo* in order to induce such an immunological response to produce antibody to protect said animal from diseases.

Further aspect of the invention relates to an immunological/vaccine formulation (composition) which, when introduced into a mammalian host, induces an immunological response in that mammal to a HLWAR77 polypeptide wherein the composition comprises a HLWAR77 polypeptide or HLWAR77 gene. The vaccine formulation may further comprise a suitable carrier. Since HLWAR77 polypeptide may be broken down in the stomach, it is preferably administered parenterally (including subcutaneous, intramuscular, intravenous, intradermal etc. injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

Screening Assays

The HLWAR77 polypeptide of the present invention may be employed in a screening process for compounds which bind the receptor and which activate (agonists) or inhibit activation of (antagonists) the receptor polypeptide of the present invention. Thus, polypeptides of the invention may also be used to assess the binding of small molecule substrates and ligands in, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These substrates and ligands may be natural substrates and ligands or may be structural or functional mimetics. See Coligan *et al.*, *Current Protocols in Immunology* 1(2):Chapter 5 (1991).

HLWAR77 polypeptides are responsible for many biological functions, including many pathologies. Accordingly, it is desirable to find compounds and drugs which stimulate HLWAR77 on the one hand and which can inhibit the function of HLWAR77 on the other hand. In general, agonists are employed for therapeutic and prophylactic purposes for such conditions as infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; diabetes, obesity; anorexia; bulimia; asthma; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hypertrophy; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette's syndrome. Antagonists may be employed for a variety of therapeutic and prophylactic purposes for such conditions as infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; diabetes, obesity; anorexia; bulimia; asthma; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; asthma; allergies; benign prostatic hypertrophy; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette's syndrome.

In general, such screening procedures involve producing appropriate cells which express the receptor polypeptide of the present invention on the surface thereof. Such cells include cells from mammals, yeast, *Drosophila* or *E. coli*. Cells expressing the receptor (or cell membrane containing the expressed receptor) are then contacted with a test compound to observe binding, or stimulation or inhibition of a functional response.

One screening technique includes the use of cells which express the receptor of this invention (for example, transfected CHO cells) in a system which measures extracellular pH or intracellular calcium changes caused by receptor activation. In this technique, compounds may be contacted with cells expressing the receptor polypeptide of the present invention. A second messenger response, e.g., signal transduction, pH changes, or changes in calcium level, is then measured to determine whether the potential compound activates or inhibits the receptor.

Another method involves screening for receptor inhibitors by determining inhibition or stimulation of receptor-mediated cAMP and/or adenylate cyclase accumulation. Such a method involves transfecting a eukaryotic cell with the receptor of this invention to express the receptor on the cell surface. The cell is then exposed to potential antagonists in the presence of the receptor of this invention. The amount of cAMP accumulation is then measured. If the potential antagonist binds the receptor, and thus inhibits receptor binding, the levels of receptor-mediated cAMP, or adenylate cyclase, activity will be reduced or increased.

Another method for detecting agonists or antagonists for the receptor of the present invention is the yeast based technology as described in U.S. Patent No. 5,482,835.

The assays may simply test binding of a candidate compound wherein adherence to the cells bearing the receptor is detected by means of a label directly or indirectly associated with the candidate compound or in an assay involving competition with a labeled competitor. Further, these assays may test whether the candidate compound results in a signal generated by activation of the receptor, using detection systems appropriate to the cells bearing the receptor at their surfaces. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed.

Further, the assays may simply comprise the steps of mixing a candidate compound with a solution containing a HLWAR77 polypeptide to form a mixture, measuring HLWAR77 activity in the mixture, and comparing the HLWAR77 activity of the mixture to a standard.

The HLWAR77 cDNA, protein and antibodies to the protein may also be used to configure assays for detecting the effect of added compounds on the production of HLWAR77 mRNA and protein in cells. For example, an ELISA may be constructed for measuring secreted or cell associated levels of HLWAR77 protein using monoclonal and polyclonal antibodies by standard methods known in the art, and this can be used to discover agents which may inhibit or enhance the production of HLWAR77 (also called antagonist or agonist, respectively) from suitably manipulated cells or tissues.

Standard methods for conducting screening assays are well understood in the art.

Examples of potential HLWAR77 antagonists include antibodies or, in some cases, oligonucleotides or proteins which are closely related to the ligand of the HLWAR77, e.g., a fragment of the ligand, or small molecules which bind to the receptor but do not elicit a response, so that the activity of the receptor is prevented.

Thus in another aspect, the present invention relates to a screening kit for identifying agonists, antagonists, ligands, receptors, substrates, enzymes, etc. for HLWAR77 polypeptides; or compounds which decrease or enhance the production of HLWAR77 polypeptides, which comprises:

- (a) a HLWAR77 polypeptide, preferably that of SEQ ID NO:2;
- (b) a recombinant cell expressing a HLWAR77 polypeptide, preferably that of SEQ ID NO:2;
- (c) a cell membrane expressing a HLWAR77 polypeptide; preferably that of SEQ ID NO: 2; or
- (d) antibody to a HLWAR77 polypeptide, preferably that of SEQ ID NO: 2.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

Prophylactic and Therapeutic Methods

This invention provides methods of treating an abnormal conditions related to both an excess of and insufficient amounts of HLWAR77 activity.

If the activity of HLWAR77 is in excess, several approaches are available. One approach comprises administering to a subject an inhibitor compound (antagonist) as hereinabove described along with a pharmaceutically acceptable carrier in an amount effective to inhibit activation by blocking binding of ligands to the HLWAR77, or by inhibiting a second signal, and thereby alleviating the abnormal condition.

In another approach, soluble forms of HLWAR77 polypeptides still capable of binding the ligand in competition with endogenous HLWAR77 may be administered. Typical embodiments of such competitors comprise fragments of the HLWAR77 polypeptide.

In still another approach, expression of the gene encoding endogenous HLWAR77 can be inhibited using expression blocking techniques. Known such techniques involve the use of antisense sequences, either internally generated or separately administered. See, for example, O'Connor, *J Neurochem* (1991) 56:560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Alternatively, oligonucleotides which form triple helices with the gene can be supplied. See, for example, Lee *et al.*, *Nucleic Acids Res* (1979) 6:3073; Cooney *et al.*, *Science* (1988) 241:456; Dervan *et al.*, *Science* (1991) 251:1360. These oligomers can be administered *per se* or the relevant oligomers can be expressed *in vivo*.

For treating abnormal conditions related to an under-expression of HLWAR77 and its activity, several approaches are also available. One approach comprises administering to a subject a therapeutically effective amount of a compound which activates HLWAR77, i.e., an agonist as described above, in combination with a pharmaceutically acceptable carrier, to thereby alleviate the abnormal condition. Alternatively, gene therapy may be employed to effect the endogenous production of HLWAR77 by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells *in vivo* and expression of the polypeptide *in vivo*. For overview of gene therapy, see Chapter 20, *Gene Therapy and other Molecular Genetic-based Therapeutic Approaches*, (and references cited therein) in Human Molecular

Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996).

Formulation and Administration

Peptides, such as the soluble form of HLWAR77 polypeptides, and agonists and antagonist peptides or small molecules, may be formulated in combination with a suitable pharmaceutical carrier. Such formulations comprise a therapeutically effective amount of the polypeptide or compound, and a pharmaceutically acceptable carrier or excipient. Such carriers include but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. Formulation should suit the mode of administration, and is well within the skill of the art. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

Preferred forms of systemic administration of the pharmaceutical compositions include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if properly formulated in enteric or encapsulated formulations, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels and the like.

The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100 µg/kg of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy" as described above. Thus, for example, cells from a subject may be engineered with a polynucleotide, such as a DNA or RNA, to encode a polypeptide *ex vivo*, and for example, by the use of a retroviral plasmid vector. The cells are then introduced into the subject.

Example 1: Mammalian Cell Expression

The receptors of the present invention are expressed in either human embryonic kidney 293 (HEK293) cells or adherent dhfr CHO cells. To maximize receptor expression, typically all 5' and 3' untranslated regions (UTRs) are removed from the receptor cDNA prior to insertion into a pCDN or pCDNA3 vector. The cells are transfected with individual receptor cDNAs by lipofectin and selected in the presence of 400 mg/ml G418. After 3 weeks of selection, individual clones are picked and expanded for further analysis. HEK293 or CHO cells transfected with the vector alone serve as negative controls. To isolate cell lines stably expressing the individual receptors, about 24 clones are typically selected and analyzed by Northern blot analysis. Receptor mRNAs are generally detectable in about 50% of the G418-resistant clones analyzed.

Example 2 Ligand bank for binding and functional assays.

A bank of over 200 putative receptor ligands has been assembled for screening. The bank comprises: transmitters, hormones and chemokines known to act via a human seven transmembrane (7TM) receptor; naturally occurring compounds which may be putative agonists for a human 7TM receptor, non-mammalian, biologically active peptides for which a mammalian counterpart has not yet been identified; and compounds not found in nature, but which activate 7TM receptors with unknown natural ligands. This bank is used to initially screen the receptor for known ligands, using both functional (i.e calcium, cAMP, microphysiometer, oocyte electrophysiology, etc, see below) as well as binding assays.

Example 3: Ligand Binding Assays

Ligand binding assays provide a direct method for ascertaining receptor pharmacology and are adaptable to a high throughput format. The purified ligand for a receptor is radiolabeled to high specific activity (50-2000 Ci/mmol) for binding studies. A determination is then made that the process of radiolabeling does not diminish the activity of the ligand towards its receptor. Assay conditions for buffers, ions, pH and other modulators, such as nucleotides, are optimized to establish a workable signal to noise ratio for both membrane and whole cell receptor sources. For these assays, specific

receptor binding is defined as total associated radioactivity minus the radioactivity measured in the presence of an excess of unlabeled competing ligand. Where possible, more than one competing ligand is used to define residual non-specific binding.

5 Example 4: Functional Assay in *Xenopus* Oocytes

Capped RNA transcripts from linearized plasmid templates encoding the receptor cDNAs of the invention are synthesized in vitro with RNA polymerases in accordance with standard procedures. In vitro transcripts are suspended in water at a final concentration of 0.2 mg/ml. Ovarian lobes are removed from adult female toads, Stage V defolliculated oocytes are obtained, and RNA transcripts (10 ng/oocyte) are injected in a 50 nl bolus using a microinjection apparatus. Two electrode voltage clamps are used to measure the currents from individual *Xenopus* oocytes in response to agonist exposure. Recordings are made in Ca²⁺ free Barth's medium at room temperature. The *Xenopus* system can be used to screen known ligands and tissue/cell extracts for activating ligands.

15 Example 5: Microphysiometric Assays

Activation of a wide variety of secondary messenger systems results in extrusion of small amounts of acid from a cell. The acid formed is largely as a result of the increased metabolic activity required to fuel the intracellular signaling process. The pH changes in the media surrounding the cell are very small but are detectable by the CYTOSENSOR microphysiometer (Molecular Devices Ltd., Menlo Park, CA). The CYTOSENSOR is thus capable of detecting the activation of a receptor which is coupled to an energy utilizing intracellular signaling pathway such as the G-protein coupled receptor of the present invention.

Example 6: Extract/Cell Supernatant Screening

A large number of mammalian receptors exist for which there remains, as yet, no cognate activating ligand (agonist). Thus, active ligands for these receptors may not be included within the ligands banks as identified to date. Accordingly, the 7TM receptor of the invention is also functionally screened (using calcium, cAMP, microphysiometer, oocyte electrophysiology, etc., functional screens) against tissue extracts to identify natural ligands. Extracts that produce positive functional responses can be sequentially subfractionated until an activating ligand is isolated and identified.

Example 7: Calcium and cAMP Functional Assays

7TM receptors which are expressed in HEK 293 cells have been shown to be coupled functionally to activation of PLC and calcium mobilization and/or cAMP stimulation or inhibition. Basal calcium levels in the HEK 293 cells in receptor-transfected or vector control cells were observed to be in the normal, 100 nM to 200 nM, range. HEK 293 cells expressing recombinant receptors are loaded with fura 2 and in a single day > 150 selected ligands or tissue/cell extracts are evaluated for agonist induced calcium mobilization. Similarly, HEK 293 cells expressing recombinant receptors are evaluated for the stimulation or inhibition of cAMP production using standard cAMP quantitation assays. Agonists presenting a calcium transient or cAMP fluctuation are tested in vector control cells to determine if the response is unique to the transfected cells expressing receptor.

Example 8: Cloning of full length sequence

An EST homologous to the 7TM superfamily sequences was identified. Oligonucleotides from these regions were designed to obtain the 5' coding region using the Marathon PCR technology. The 5' oligos were (AAAGACTGAAGCT-GCGACAGATATTC (SEQ ID NO:5) AND CCTGGACCAATCCACTGATCTTGC (SEQ ID NO:6)). Fragments of approximately 1200 bp (5' fragment) were obtained and subcloned into PCR 2.1 vector (Invitrogene) and sequenced. Sequence analysis showed an open reading frame starting with nucleotide 348(ATG) and ending at nucleotide 1610 (TGA). The coding region is 1263 nucleotides which encodes a protein of 421 amino acids. The protein is predicted to have a molecular weight of approximately 46,310.

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

SEQUENCE LISTING

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(1) GENERAL INFORMATION

(i) APPLICANT: SmithKline Beecham Corporation

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(ii) TITLE OF THE INVENTION: 7TM RECEPTOR HLWAR77

(iii) NUMBER OF SEQUENCES: 6

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(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: SmithKline Beecham, Corporate Intellectual
Property

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(B) STREET: Two New Horizons Court

(C) CITY: Brentford

(D) STATE: Middlesex

(E) COUNTRY: United Kingdom

25

(F) ZIP: TW8 9EP

(v) COMPUTER READABLE FORM:

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(A) MEDIUM TYPE: Diskette

(B) COMPUTER: IBM Compatible

(C) OPERATING SYSTEM: DOS

(D) SOFTWARE: FastSEQ for Windows Version 2.0

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(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: TO BE ASSIGNED

(B) FILING DATE: 13-JAN-1998

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(C) CLASSIFICATION: UNKNOWN

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 60/049,332

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(B) FILING DATE: 11-JUN-1997

(A) APPLICATION NUMBER: 60/067,253

(B) FILING DATE: 02-DEC-1997

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(viii) ATTORNEY/AGENT INFORMATION:

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(A) NAME: CONNELL, Anthony Christopher
(B) REGISTRATION NUMBER: 5630 & 26758
(C) REFERENCE/DOCKET NUMBER: GH70074

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: +44 1279 644 395
(B) TELEFAX: +44 181 975 6294
(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1888 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCATCCTAAT	ACGACTCACT	ATAGGGCTCG	AGCGGCCGCC	CGGGCAGGTT	CTCGGCTCAC	60
TGCAAGCTCC	ACCTCCTGGG	TTCACGCTAT	TCTCTGCCT	CAGCCTCCTG	AGTAGCTGGG	120
ACTACAGGCG	CCCGCCACCA	CGCCTGGCTA	ATTTTTTTGT	ATTTTATAGTA	GGGACGGCGT	180
TTCACTGTGT	TAGCCAGAT	GGTCTGCGTC	TCCCGACCTC	GTGATCCACC	CACCTCGGCT	240
TCCCAAAGTG	CTGGGATTAC	AGGCGTGAGC	CACCGCGCCC	GGCCAATTTT	CTTTCTTAAT	300
TGCCTCTGCC	CACCTCTTCT	CTTCTGCTTC	CATATTACAG	GTTTCATCATG	AATGAGAAAT	360
GGGACACAAA	CTCTTCAGAA	AACTGGCATC	CCATCTGGAA	TGTCAATGAC	ACAAAGCATC	420
ATCTGTACTC	AGATATTAAT	ATTACCTATG	TGAACTACTA	TCTTCACCAG	CCTCAAGTGG	480
CAGCAATCTT	CATTATTTCC	TACTTTCTGA	TCTTCTTTTT	GTGCATGATG	GGAAATACTG	540
TGGTTTGCTT	TATTGTAATG	AGGAACAAAC	ATATGCACAC	AGTCACTAAT	CTCTTCATCT	600
TAAACCTGGC	CATAAGTGAT	TTACTAGTTG	GCATATTCTG	CATGCCTATA	ACACTGCTGG	660
ACAATATTAT	AGCAGGATGG	CCATTTGGAA	ACACGATGTG	CAAGATCAGT	GGATTGGTCC	720
AGGGAATATC	TGTCGCAGCT	TCAGTCTTTA	CGTTAGTTGC	AATTGCTGTA	GATAGGTTCC	780
AGTGTGTGGT	CTACCCTTTT	AAACCAAAGC	TCACTATCAA	GACAGCGTTT	GTCATTATTA	840
TGATCATCTG	GGTCCTAGCC	ATCACCATTA	TGTCTCCATC	TGCAGTAATG	TTACATGTGC	900
AAGAAGAAAA	ATATTACCGA	GTGAGACTCA	ACTCCCAGAA	TAAAACCAGT	CCAGTCTACT	960
GGTGCCGGGA	AGACTGGCCA	AATCAGGAAA	TGAGGAAGAT	CTACACCACT	GTGCTGTTTG	1020
CCAACATCTA	CCTGGCTCCC	CTCTCCCTCA	TTGTCATCAT	GTATGGAAGG	ATTGGAATTT	1080
CACCTTTQAG	GGCTGCAGTT	CCTCACACAG	GCAGGAAGAA	CCAGGAGCAG	TGGCACGTGG	1140
TGTCCAGGAA	GAAGCAGAAG	ATCATTAAAG	TGCTCCTGAT	TGTGGCCCTG	CTTTTATTTC	1200

TCTCATGGCT GCCCCTGTGG ACTCTAATGA TGCTCTCAGA CTACGCTGAC CTTTCTCCAA 1260
 ATGAACTGCA GATCATCAAC ATCTACATCT ACCCTTTTGC AACTGGCTG GCATTCGGCA 1320
 ACAGCAGTGT CAATCCCATC ATTTATGGTT TCTTCAACGA GAATTTCCGC CGTGGTTTCC 1380
 AAGAAGCTTT CCAGCTCCAG CTCTGCCAAA AAAGAGCAAA GCCTATGGAA GCTTATGCCC 1440
 TAAAAGCTAA AAGCCATGTG CTCATAAACA CATCTAATCA GCTTGTCCAG GAATCTACAT 1500
 TTCAAAACCC TCATGGGGAA ACCTTGCTTT ATAGGAAAAG TGCTGAAAAA CCCCACAGG 1560
 AATTAGTGAT GGAAGAATTA AAAGAACTA CTAACAGCAG TGAGATTAA AAAGAGCTAG 1620
 TGTGATAATC CTAACCTCTAC TACGCATTAT ATATTTAAAT CCATTGCTTT TTGTGGCTTT 1680
 GCACTTCAAA TTTTTCAAAG AATGTTCTAA ATAAAACATT TACTGAAAGC CCTCTCTGGC 1740
 AAAAAAATTA AAAATAAACA AAAATGGTCA TAAGATCATA AACAATCTTA TGTTGTATAA 1800
 AAATACGTAG AGTGACTTAG ACATGTTTGC ATGAATAAAT ATATTTCTAG AGAACAGTTA 1860
 AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA 1888

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 420 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Asn Glu Lys Trp Asp Thr Asn Ser Ser Glu Asn Trp His Pro Ile
 1 5 10 15
 Trp Asn Val Asn Asp Thr Lys His His Leu Tyr Ser Asp Ile Asn Ile
 20 25 30
 Thr Tyr Val Asn Tyr Tyr Leu His Gln Pro Gln Val Ala Ala Ile Phe
 35 40 45
 Ile Ile Ser Tyr Phe Leu Ile Phe Phe Leu Cys Met Met Gly Asn Thr
 50 55 60
 Val Val Cys Phe Ile Val Met Arg Asn Lys His Met His Thr Val Thr
 65 70 75 80
 Asn Leu Phe Ile Leu Asn Leu Ala Ile Ser Asp Leu Leu Val Gly Ile
 85 90 95
 Phe Cys Met Pro Ile Thr Leu Leu Asp Asn Ile Ile Ala Gly Trp Pro
 100 105 110
 Phe Gly Asn Thr Met Cys Lys Ile Ser Gly Leu Val Gln Gly Ile Ser
 115 120 125
 Val Ala Ala Ser Val Phe Thr Leu Val Ala Ile Ala Val Asp Arg Phe

130 135 140
 Gln Cys Val Val Tyr Pro Phe Lys Pro Lys Leu Thr Ile Lys Thr Ala
 145 150 155 160
 Ph Val Ile Ile Met Ile Ile Trp Val Leu Ala Ile Thr Ile Met Ser
 165 170 175
 Pro Ser Ala Val Met Leu His Val Gln Glu Glu Lys Tyr Tyr Arg Val
 180 185 190
 Arg Leu Asn Ser Gln Asn Lys Thr Ser Pro Val Tyr Trp Cys Arg Glu
 195 200 205
 Asp Trp Pro Asn Gln Glu Met Arg Lys Ile Tyr Thr Thr Val Leu Phe
 210 215 220
 Ala Asn Ile Tyr Leu Ala Pro Leu Ser Leu Ile Val Ile Met Tyr Gly
 225 230 235 240
 Arg Ile Gly Ile Ser Leu Phe Arg Ala Ala Val Pro His Thr Gly Arg
 245 250 255
 Lys Asn Gln Glu Gln Trp His Val Val Ser Arg Lys Lys Gln Lys Ile
 260 265 270
 Ile Lys Met Leu Leu Ile Val Ala Leu Leu Phe Ile Leu Ser Trp Leu
 275 280 285
 Pro Leu Trp Thr Leu Met Met Leu Ser Asp Tyr Ala Asp Leu Ser Pro
 290 295 300
 Asn Glu Leu Gln Ile Ile Asn Ile Tyr Ile Tyr Pro Phe Ala His Trp
 305 310 315 320
 Leu Ala Phe Gly Asn Ser Ser Val Asn Pro Ile Ile Tyr Gly Phe Phe
 325 330 335
 Asn Glu Asn Phe Arg Arg Gly Phe Gln Glu Ala Phe Gln Leu Gln Leu
 340 345 350
 Cys Gln Lys Arg Ala Lys Pro Met Glu Ala Tyr Ala Leu Lys Ala Lys
 355 360 365
 Ser His Val Leu Ile Asn Thr Ser Asn Gln Leu Val Gln Glu Ser Thr
 370 375 380
 Phe Gln Asn Pro His Gly Glu Thr Leu Leu Tyr Arg Lys Ser Ala Glu
 385 390 395 400
 Lys Pro Gln Gln Glu Leu Val Met Glu Glu Leu Lys Glu Thr Thr Asn
 405 410 415
 Ser Ser Glu Ile
 420

(2) INFORMATION FOR SEQ ID NO:3:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1508 base pairs

(B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AGTGAACCGT	CAGATCGCCT	GGAGACGCCA	TCCACGCTGT	TTTGACCTCC	ATAGAAGACA	60
CCGGGACCGA	TCCAGCCTCC	GGACTCTAGC	CTAGGCCGCG	GGACGGATAA	CAATTTTACA	120
CAGGAAACAG	CTATGACCAC	TAGGCTTTTG	CAAAAAGCTA	TTTAGGTGAC	ACTATAGAAG	180
GTACGCCTGC	AGGTACCGGT	CCGGAATTCC	CGGGTCGACC	CACGCGTCCG	CATAAGTGAT	240
TTACTAGTTG	GCATATTCTG	CATGCCTATA	ACACTGCTGG	ACAATATTAT	AGCAGGATGG	300
CCATTTGGAA	ACACGATGTG	CAAGATCAGT	GGATTGGTCC	AGGGAATATC	TGTCGCAGCT	360
TCAGTCTTTA	CGTTAGTTGC	AATTGCTGTA	GATAGGTTCC	AGTGTGTGGT	CTACCCTTTT	420
AAACCAAAGC	TCATATCAA	GACAGCGTTT	GTCATTATTA	TGATCATCTG	GGTCCTAGCC	480
ATCACCATTA	TGCTCTCATC	TGCAGTAATG	TTACATGTGC	AAGAAGAAAA	ATATTACCGA	540
GTGAGACTCA	ACTCCAGAA	TAAAACCAGT	CCAGTCTACT	GGTGCCGGGA	AGACTGGCCA	600
AATCAGGAAA	TGAGGAAGAT	CTACACCACT	GTGCTGTTTG	CCAACATCTA	CCTGGCTCCC	660
CTCTCCCTCA	TTGTCATCAT	STATGGAAGG	ATTGGAATTT	CACTCTTCAG	GGCTGCAGTT	720
CCTCACACAG	GCAGGAAGAA	CCAGGAGCAG	TGGCACGTGG	TGTCCAGGAA	GAAGCAGAAG	780
ATCATTAAAG	TGCTCCTGAT	TGTGGCCCTG	CTTTTTATTG	TCTCATGGCT	GCCCCGTGTG	840
ACTCTAATGA	TGCTCTCAGA	CTACGCTGAC	CTTTCTCCAA	ATGAACTGCA	GATCATCAAC	900
ATCTACATCT	ACCCTTTTCG	ACACTGGCTG	GCATTGGGCA	ACAGCAGTGT	CAATCCCATC	960
ATTTATGGTT	TCTTCAACGA	GAATTTCCGC	CGTGGTTTCC	AAGAAGCTTT	CCAGCTCCAG	1020
CTCTGCCAAA	AAAGAGCAAA	GCCTATGGAA	GCTTATGCCC	TAAAAGCTAA	AAGCCATGTG	1080
CTCATAAACA	CATCTAATCA	GCTTGTCCAG	GAATCTACAT	TTCAAACCC	TCATGGGGAA	1140
ACCTTGCTTT	ATAGGAAAAG	TGCTGAAAAA	CCCCAACAGG	AATTAGTGAT	GGAAGAATTA	1200
AAAGAACTA	CTAACAGCAG	TGAGATTTAA	AAAGAGCTAG	TGTGATAATC	CTAACTCTAC	1260
TACGCATTAT	ATATTTAAAT	CCATTGCTTT	TTGTGGCTTT	GCACTTCAA	TTTTTCAAAG	1320
AATGTTCTAA	ATAAACATT	TACTGAAAGC	CCTCTCTGGC	AAAAAATTA	AAAATAAACA	1380
AAAATGGTCA	TAAGATCATA	AACAATCTTA	TGTTGTATAA	AAATACGTAG	AGTGACTTAG	1440
ACATGTTTGC	ATGAATAAAT	ATATTTCTAG	AGAACAGTTA	AAAAAAAAAA	AAAAAAAAAA	1500
AAAAAAAAAA						1508

(2) INFORMATION FOR SEQ ID NO:4:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 336 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Pro Arg Val Arg Ile Ser Asp Leu Leu Val Gly Ile Phe Cys Met Pro
 1           5           10           15
Ile Thr Leu Leu Asp Asn Ile Ile Ala Gly Trp Pro Phe Gly Asn Thr
20           25           30
Met Cys Lys Ile Ser Gly Leu Val Gln Gly Ile Ser Val Ala Ala Ser
35           40           45
Val Phe Thr Leu Val Ala Ile Ala Val Asp Arg Phe Gln Cys Val Val
50           55           60
Tyr Pro Phe Lys Pro Lys Leu Thr Ile Lys Thr Ala Phe Val Ile Ile
65           70           75           80
Met Ile Ile Trp Val Leu Ala Ile Thr Ile Met Ser Pro Ser Ala Val
85           90           95
Met Leu His Val Gln Glu Glu Lys Tyr Tyr Arg Val Arg Leu Asn Ser
100          105          110
Gln Asn Lys Thr Ser Pro Val Tyr Trp Cys Arg Glu Asp Trp Pro Asn
115          120          125
Gln Glu Met Arg Lys Ile Tyr Thr Thr Val Leu Phe Ala Asn Ile Tyr
130          135          140
Leu Ala Pro Leu Ser Leu Ile Val Ile Met Tyr Gly Arg Ile Gly Ile
145          150          155          160
Ser Leu Phe Arg Ala Ala Val Pro His Thr Gly Arg Lys Asn Gln Glu
165          170          175
Gln Trp His Val Val Ser Arg Lys Lys Gln Lys Ile Ile Lys Met Leu
180          185          190
Leu Ile Val Ala Leu Leu Phe Ile Leu Ser Trp Leu Pro Leu Trp Thr
195          200          205
Leu Met Met Leu Ser Asp Tyr Ala Asp Leu Ser Pro Asn Glu Leu Gln
210          215          220
Ile Ile Asn Ile Tyr Ile Tyr Pro Phe Ala His Trp Leu Ala Phe Gly
225          230          235          240
Asn Ser Ser Val Asn Pro Ile Ile Tyr Gly Phe Phe Asn Glu Asn Phe
245          250          255
Arg Arg Gly Phe Gln Glu Ala Phe Gln Leu Gln Leu Cys Gln Lys Arg
260          265          270
Ala Lys Pro Met Glu Ala Tyr Ala Leu Lys Ala Lys Ser His Val Leu
275          280          285
Ile Asn Thr Ser Asn Gln Leu Val Gln Glu Ser Thr Phe Gln Asn Pro
290          295          300

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His Gly Glu Thr Leu Leu Tyr Arg Lys Ser Ala Glu Lys Pro Gln Gln
 305 310 315 320
 5 Glu Leu Val Met Glu Glu Leu Lys Glu Thr Thr Asn Ser Ser Glu Ile
 325 330 335

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AAAGACTGAA GCTGCGACAG ATATTC

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(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CCTGGACCAA TCCACTGATC TTGC

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Claims

1. An isolated polynucleotide comprising a nucleotide sequence that has at least 80% identity over its entire length to a nucleotide sequence encoding the HLWAR77 polypeptide of SEQ ID NO:2; or a nucleotide sequence complementary to said isolated polynucleotide.
2. The polynucleotide of claim 1 wherein said polynucleotide comprises the nucleotide sequence contained in SEQ ID NO:1 encoding the HLWAR77 polypeptide of SEQ ID NO:2.
3. The polynucleotide of claim 1 wherein said polynucleotide comprises a nucleotide sequence that is at least 80%

identical to that of SEQ ID NO: 1 over its entire length.

4. The polynucleotide of claim 3 which is polynucleotide of SEQ ID NO: 1.
- 5 5. The polynucleotide of claim 1 which is DNA or RNA.
6. A DNA or RNA molecule comprising an expression system, wherein said expression system is capable of producing a HLWAR77 polypeptide comprising an amino acid sequence, which has at least 80% identity with the polypeptide of SEQ ID NO:2 when said expression system is present in a compatible host cell.
- 10 7. A host cell comprising the expression system of claim 6.
8. A process for producing a HLWAR77 polypeptide comprising culturing a host of claim 7 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture.
- 15 9. A process for producing a cell which produces a HLWAR77 polypeptide thereof comprising transforming or transfecting a host cell with the expression system of claim 6 such that the host cell, under appropriate culture conditions, produces a HLWAR77 polypeptide.
- 20 10. A HLWAR77 polypeptide comprising an amino acid sequence which is at least 80% identical to the amino acid sequence of SEQ ID NO:2 over its entire length.
11. The polypeptide of claim 10 which comprises the amino acid sequence of SEQ ID NO:2.
- 25 12. An antibody immunospecific for the HLWAR77 polypeptide of claim 10.
13. A method for the treatment of a subject in need of enhanced activity or expression of HLWAR77 polypeptide of claim 10 comprising:
 - 30 (a) administering to the subject a therapeutically effective amount of an agonist to said receptor; and/or
 - (b) providing to the subject an isolated polynucleotide comprising a nucleotide sequence that has at least 80% identity to a nucleotide sequence encoding the HLWAR77 polypeptide of SEQ ID NO:2 over its entire length; or a nucleotide sequence complementary to said nucleotide sequence in a form so as to effect production of said receptor activity *in vivo*.
- 35 14. A method for the treatment of a subject having need to inhibit activity or expression of HLWAR77 polypeptide of claim 10 comprising:
 - 40 (a) administering to the subject a therapeutically effective amount of an antagonist to said receptor; and/or
 - (b) administering to the subject a nucleic acid molecule that inhibits the expression of the nucleotide sequence encoding said receptor; and/or
 - (c) administering to the subject a therapeutically effective amount of a polypeptide that competes with said receptor for its ligand.
- 45 15. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of HLWAR77 polypeptide of claim 10 in a subject comprising:
 - (a) determining the presence or absence of a mutation in the nucleotide sequence encoding said HLWAR77 polypeptide in the genome of said subject; and/or
 - 50 (b) analyzing for the presence or amount of the HLWAR77 polypeptide expression in a sample derived from said subject.
16. A method for identifying agonists to HLWAR77 polypeptide of claim 10 comprising:
 - 55 (a) contacting a cell which produces a HLWAR77 polypeptide with a candidate compound; and
 - (b) determining whether the candidate compound effects a signal generated by activation of the HLWAR77 polypeptide.

17. An agonist identified by the method of claim 16.

18. The method for identifying antagonists to HLWAR77 polypeptide of claim 10 comprising:

- 5 (a) contacting a cell which produces a HLWAR77 polypeptide with an agonist; and
 (b) determining whether the signal generated by said agonist is diminished in the presence of a candidate compound.

19. An antagonist identified by the method of claim 18.

20. A recombinant host cell produced by a method of Claim 9 or a membrane thereof expressing a HLWAR77 polypeptide.

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